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PATENT
ATTORNEY DOCKET NO. 50195/023003

Certificate of Mailing: Date of Deposit: December 1, 2008

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as **first class mail** with sufficient postage on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, Attention: Certificate of Correction Branch.

Joseph Povec
Printed name of person mailing correspondence

Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Robl et al.	Confirmation No.:	4828
Serial No.:	10/705,519	Art Unit:	1632
Filed:	November 10, 2003	Examiner:	Deborah Crouch
Patent No.:	7,429,690	Customer No.:	21559
Issued:	September 30, 2008		
Title:	TRANSGENIC BOVINES HAVING REDUCED PRION PROTEIN PRODUCTION		

Certificate
DEC 0 5 2008
of Correction

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention: Certificate of Correction Branch

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.323

Applicants hereby request that a Certificate of Correction be issued in the patent identified above. The errors to be corrected are of a minor, typographical nature, occurred in good faith, and are described in detail on the enclosed PTO Form 1050.

Applicants note that the error to be corrected at column 157, line 11 occurs within issued claim 11. Applicants submit that the term "homologous" mutation at column 157, line 11 should be replaced with the term "homozygous" mutation. Support for this correction is found in the version of claim 32 filed with the Reply to Office Action in this case on February 11, 2008. On February 11, 2008, pending claim 32 (corresponding to

issued claim 11) recited the term "homozygous" mutation. A copy of the February 11, 2008 Reply to Office Action downloaded from the PAIR ImageFile Wrapper is enclosed (Exhibit A).

Following submission of this Reply, the Examiner discussed the pending claims in an interview with Applicants on May 30, 2008. The corresponding Interview Summary downloaded from the PAIR ImageFile Wrapper (Exhibit B) does not discuss amendment of the term "homozygous" mutation to "homologous" mutation, and therefore the introduction of the term "homologous" mutation in the Examiner's Amendment included with the Notice of Allowability on June 11, 2008 was made in error. Applicants respectfully request correction of the term "homologous" mutation at column 157, line 11 to "homozygous" mutation, as indicated in the enclosed Form PTO 1050. No new matter is added by the requested corrections.

Enclosed is a check for $100.00 in payment of the fee set forth in 37 C.F.R. § 1.20(a).

If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 01 December 2008

Karen L. Elbing, Ph.D.
Reg. No. 35,238

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

Exhibit B

Interview Summary	Application No. 10/705,519	Applicant(s) ROBL ET AL.	
	Examiner Deborah Crouch, Ph.D.	Art Unit 1632	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Deborah Crouch, Ph.D.*. (3)_____.

(2) *Karen Elbing*. (4)_____.

Date of Interview: *30 May 2008*.

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No.
If Yes, brief description: _____.

Claim(s) discussed: *1,3,4,7,9-25,28 and 35-37*.

Identification of prior art discussed: _____.

Agreement with respect to the claims f)☒ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *Ms. Elbing agreed to insert "transcriptional termination sequence" in claims 1,25,32 and 37, change "hemizygous" to "heterogzygous" in claims 4 and 38; and to limit claims 37 to fibroblast for allowance. The agreement was made without prejudice towards further prosecution in a continuing application.*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

_____ Examiner's signature, if required

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. 7,429,690 B2
DATED September 30, 2008
INVENTORS Robl et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Cover Sheet, Under ABSTRACT, replace "encephalopy" with --encephalopathy--.

On Page 3, Under References Cited, Under OTHER PUBLICATIONS, second column, reference Griffiths et al., replace "Role in the Nervous Sytem" with --Role in the Nervous System--.

On Page 4, Under References Cited, Under OTHER PUBLICATIONS, second column, reference Hamers-Casterman et al., replace "Naturally Occuring Antibodies" with --Naturally Occurring Antibodies--.

On Page 4, Under References Cited, Under OTHER PUBLICATIONS, second column, reference Park et al., replace "Transfer Embyros Derived" with --Transfer Embryos Derived--.

Column 1, Line 19, replace "encephalopy" with --encephalopathy--.

Column 2, Line 36, replace "PC" with --PCT--.

Column 6, Line 22, replace "immunogloblulin" with --immunoglobulin--.

Column 10, Line 22, replace "ooctye" with --oocyte--.

Column 11, Line 60, replace "nuleic" with --nucleic--.

MAILING ADDRESS OF SENDER:
Karen L. Elbing, Ph.D.
Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Customer No.: 21559

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. 7,429,690 B2
DATED September 30, 2008
INVENTORS Robl et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 21, Line 20, replace "polynucloetide" with
--polynucleotide--.

Column 28, Line 66, replace "tranfection" with --transfection--.

Column 31, Line 13, replace "aicd." with --acid.--.

Column 32, Line 11, replace "ooctye" with --oocyte--.

Column 33, Line 16, replace "wouldl" with --would--.

Column 34,
Line 23, replace "apuro" with --a puro--.
Line 25, replace "inearized" with --linearized--.

Column 36, Line 40, replace "wase" with --was--.

Column 41, Line 52, replace "cytochalacin" with --cytochalasin--.

Column 50,
Line 12, replace "phycoerytherin" with --phycoerythrin--.
Line 27, replace "bove" with --bovine--.

Column 54, Line 38, replace "hemizgously" with --hemizygously--.

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Clark & Elbing LLP
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DATED September 30, 2008
INVENTORS Robl et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 59,
Line 5, replace "therefor" with --therefore--.
Line 18, replace "immunoglobulon" with --immunoglobulin--.

Column 65, Line 16, replace "transfering" with --transferring--.

Column 73,
Line 2, replace "fibrolast" with --fibroblast--.
Line 4, replace "pbluescript" with --Pbluescript--.

Column 82, Line 53, replace "coverlips" with --coverslips--.

Column 84, Line 35, replace "embyros" with --embryos--.

Column 86, Line 40, replace "nulear" with --nuclear--.

Column 87, Line 53, replace "nulear" with --nuclear--.

Column 91, Line 51, replace "enucleatation" with --enucleation--.

Column 96, Line 30, replace "permeablized" with --permeabilized--.

Column 97, in Table 7, replace "pemeabilized" with --permeabilized--.

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Clark & Elbing LLP
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CERTIFICATE OF CORRECTION

PATENT NO. 7,429,690 B2
DATED September 30, 2008
INVENTORS Robl et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 98, Line 25, replace "pippette." with --pipette.--.

Column 99,
Line 52, replace "mitotsis" with --mitosis--.
Line 59, replace "ooctyes" with --oocytes--.

Column 100, Line 30, replace "ooctyes" with --oocytes--.

Column 102, Line 3, replace "precentage" with --percentage--.

Column 105, Line 31, replace "parthenogenically" with --parthenogenetically--.

Column 113,
Line 19, replace "permeablized" with --permeabilized--.
Line 25, replace "successfuly" with --successfully--.

Column 115,
Line 10, replace "mircoseconds" with --microseconds--.
Line 50, replace "epinepherine" with --epinephrine--.

Column 116, Line 8, replace "blatomeres" with --blastomeres--.

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Karen L. Elbing, Ph.D.
Clark & Elbing LLP
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CERTIFICATE OF CORRECTION

PATENT NO. 7,429,690 B2
DATED September 30, 2008
INVENTORS Robl et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 118,
Line 35, replace "Coryunebacterium" with --Corynebacterium--.
Line 37, replace "botulinium" with --botulinum--.

Column 119, Line 9, replace "Intraveneous" with --Intravenous--.

Column 120,
Line 39, replace "permeablizing" with --permeabilizing--.
Line 41, replace "tranfer" with --transfer--.

Column 157, Line 11, replace "homologous" with --homozygous--.

MAILING ADDRESS OF SENDER:
Karen L. Elbing, Ph.D.
Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Customer No.: 21559

PATENT NO. 7,429,690 B2
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DEC - 5 2008

Exhibit A

PATENT
ATTORNEY DOCKET NO. 50195/023003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	James M. Robl et al.	Confirmation No.:	4828
Serial No.:	10/705,519	Art Unit:	1632
Filed:	November 10, 2003	Examiner:	Deborah Crouch
Customer No.:	21559		
Title:	TRANSGENIC UNGULATES HAVING REDUCED PRION PROTEIN ACTIVITY AND USES THEREOF		

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO OFFICE ACTION

In reply to the Office action mailed on August 9, 2007 in connection with the above-referenced case, Applicants provide the following amendments and remarks.

AMENDMENTS

Kindly amend the application as follows.

AMENDMENTS TO THE CLAIMS

1. (Previously presented) A bovine whose genome comprises a non-naturally occurring mutation in one or both alleles of an endogenous prion nucleic acid, wherein said bovine exhibits reduced functional prion production.

2-3. (Cancelled).

4. (Original) The bovine of claim 1, wherein said mutation is hemizygous.

5. (Previously presented) The bovine of claim 1, wherein said mutation is homozygous and said bovine exhibits no functional prion production.

6. (Original) The bovine of claim 1, wherein said mutation comprises an insertion of a positive selection marker into a prion nucleic acid.

7. (Original) The bovine of claim 1, wherein said mutation comprises an insertion of a STOP codon into a prion nucleic acid.

8. (Original) The bovine of claim 1, wherein said mutation comprises a deletion of one or more nucleotides in a prion nucleic acid.

9. (Withdrawn) The bovine of claim 1, comprising one or more nucleic acids comprising one or more transgenes and expressing an mRNA or protein encoded by said transgene(s).

10. (Withdrawn) The bovine of claim 1, comprising one or more nucleic acids comprising all or part of a xenogenous immunoglobulin (Ig) gene which undergoes rearrangement and expresses more than one xenogenous Ig molecule.

11. (Withdrawn) The bovine of claim 10, comprising one or more nucleic acids encoding a xenogenous antibody.

12. (Withdrawn) The bovine of claim 11, wherein said xenogenous antibody is a human antibody.

13. (Withdrawn) The bovine of claim 12, wherein said antibody is expressed in serum and/or milk.

14. (Withdrawn) The bovine of claim 1, comprising a mutation that reduces the expression of an endogenous antibody.

15. (Withdrawn) The bovine of claim 14, wherein said mutation reduces the expression of functional IgM heavy chain.

16. (Withdrawn) The bovine of claim 15, wherein said mutation substantially eliminates the expression of functional IgM heavy chain.

17. (Withdrawn) The bovine of claim 14, wherein said mutation reduces the expression of functional Ig light chain.

18. (Withdrawn) The bovine of claim 17, wherein said mutation substantially eliminates the expression of functional Ig light chain.

19. (Withdrawn) The bovine of claim 14, wherein said mutation reduces the expression of functional IgM heavy chain and functional Ig light chain.

20. (Withdrawn) The bovine of claim 19, wherein said mutation substantially eliminates the expression of functional IgM heavy chain and functional Ig light chain.

21. (Withdrawn) The bovine of claim 1, comprising a mutation in one or both alleles of an endogenous nucleic acid encoding alpha-(1,3)-galactosyltransferase.

22. (Withdrawn) The bovine of claim 1, comprising a mutation in one or both alleles of an endogenous nucleic acid encoding J chain.

23. (Withdrawn) The bovine of claim 1, comprising a nucleic acid encoding an exogenous J chain.

24. (Withdrawn) The bovine of claim 23, wherein said J chain is a human J chain.

25. (Currently amended) An isolated bovine cell comprising a non-naturally occurring mutation in one or both alleles of an endogenous prion nucleic acid, wherein said bovine cell exhibits reduced functional prion production.

26-27. (Cancelled).

28. (Original) The cell of claim 25, wherein said mutation is hemizygous.

29. (Previously Presented) The cell of claim 25, wherein said mutation is homozygous and said bovine cell exhibits no functional prion production.

30. (Original) The cell of claim 25, wherein said cell is a fetal fibroblast.

31. (Original) The cell of claim 25, wherein said cell is a B-cell.

32. (Previously presented) A method for producing an isolated transgenic bovine cell having reduced expression of functional prion protein, comprising

(a) introducing a first prion gene targeting vector into a bovine cell under conditions that allow homologous recombination between said first vector and a first allele of an endogenous prion nucleic acid in said cell, thereby introducing a hemizygous mutation in said cell;

(b) isolating said bovine cell containing hemizygous mutation; and

(c) introducing a second prion gene targeting vector having a different selectable marker than said first vector into said bovine cell of step (b) under conditions that allow homologous recombination between said second vector and a second allele of an endogenous prion nucleic acid in said cell, thereby introducing a homozygous mutation in said bovine cell.

33-34. (Cancelled).

35. (Original) The method of claim 32, wherein said cell is a bovine fibroblast.

36. (Original) The method of claim 35, wherein said cell is a bovine fetal fibroblast.

37. (Previously presented) A method for producing a transgenic bovine having reduced expression of functional prion protein, said method comprising the steps of:

(a) inserting a diploid permeabilized cell into an enucleated metaphase II oocyte, wherein said cell comprises a first non-naturally occurring mutation in an endogenous prion nucleic acid; and

(b) transferring said oocyte or an embryo formed from said oocyte into the uterus of a host bovine under conditions that allow said oocyte or said embryo to develop into a

fetus, wherein the genome of said fetus comprises said non-naturally occurring mutation in said endogenous prion nucleic acid and wherein said fetus exhibits reduced functional prion production.

38. (Original) The method of claim 37, wherein said fetus develops into a viable offspring.

39. (Cancelled).

REMARKS

The Office Action

Claims 1, 4-25, 28-32, and 35-38 are pending. Claims 1 and 7 stand rejected for lack of enablement. Claims 25 and 28-31 stand rejected for indefiniteness. Claims 1, 4, 6, 8, 25, 28-30, 32, 35, and 36 stand rejected for anticipation by Good et al. (U.S. Patent Publication No. 2002/0069423; hereafter "Good"), with reference to Bondioli et al. (Mol. Reproduct. Develop., 2001, 60:189 ; hereafter "Bondioli"), Dai et al. (Nature Biotech., 2002, 20:251; hereafter "Dai"), and Zheng et al. (Proc. Natl. Acad. Sci. USA, 1991, 88:8067). Claims 1 and 7 stand rejected for obviousness over Good in view of Muramoto et al. (Nature Med. 1997, 3:750; hereafter "Muramoto"). Claims 25, 31, 37, and 38 stand rejected for obviousness over Good in view of Collas et al. (U.S. Patent Publication No. 2003/0046722; hereafter "Collas"). These rejections are addressed below.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1 and 7 stand rejected for lack of enablement. According to the Office, "The claims are not enabled because a human prion disease is known in the art to be associated with an improper termination codon, an amber codon, inserted by a naturally occurring mutation of the human endogenous prion gene [as reported by Muramoto]." Based on this disclosure in Muramoto, the Office concludes that "it is unpredictable that a bovine produced through the insertion of a STOP codon in its prion gene will be prion disease free." Applicants traverse this rejection.

The Muramoto reference teaches that an amber mutation in a *human* prion gene still results in disease. The reference further teaches (as acknowledged by the Office in the § 103 rejection) that *mice* having the same amber mutation *are* prion disease free. Muramoto thus teaches that the effect of a prion amber mutation in one species is not necessarily predictive of the effect of the same mutation in another. Significantly, Muramoto does not provide any experimental evidence or commentary relating to

bovines, as instantly claimed. Applicants therefore submit that the reference is not directly relevant to the predictability of reducing prion gene expression in bovines.

Moreover, this rejection may be withdrawn as Applicants have demonstrated success in using the methods described in the present specification to produce bovines having inserted STOP codons in their prion genes and showing that these bovines do not express prion proteins. As stated by Dr. Kuroiwa in his Declaration of June 2007, to produce prion knockout bovines, a male Holstein primary fetal fibroblast line was transfected with first and second KO vectors (pBPrP(H)KOneo and pBPrP(H)KOpuro vectors) to sequentially disrupt the two alleles of the PrP gene by insertion of STOP codons. Colonies identified to be $PrP^{-/-}$ were used for embryonic cloning to generate recloned fetuses. From these fetuses, fibroblast cell lines were established and confirmed to be homozygous $PrP^{-/-}$ by targeting event-specific PCR analysis and *prion-negative by PCR analysis*. Three of these $PrP^{-/-}$ fetal cell lines were recloned to produce calves. These experiments demonstrate that the methods described in the specification may be, and were, employed to successfully produce living PrP knockout bovines having STOP codons inserted in their prion genes. Further, prion protein expression in the living bovines was not detectable by Western blot.

Thus, all evidence of record relevant to bovines indicates that prion-free bovines can be produced using targeted insertion of a STOP codon. Reconsideration and withdrawal of this rejection is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 25 and 28-31 stand rejected for indefiniteness. In the action, the Office has suggested that inclusion of the term "isolated" would obviate this rejection. Applicants have amended claim 25 as suggested, and the rejection may be withdrawn.

Rejection under 35 U.S.C. § 102

Claims 1, 4, 6, 8, 25, 28-30, 32, 35, and 36 remain rejected for anticipation by Good. This rejection is respectfully traversed. As stated in the M.P.E.P., "The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation" (M.P.E.P. § 2121.01; citations omitted). Applicants maintain that the anticipation rejection in this case should be withdrawn because Good is not an enabling disclosure for the production of bovines or bovine cells having reduced prion production.

The Good Disclosure

As was noted by Dr. Kuroiwa in his Declaration of September 2006,[1] Good provides three potential methods for producing prion knockout cows. Two of these methods were attempted experimentally. The first is described at paragraph [0122], where Good describes an attempt to isolate genomic sequences of the PrP gene in order to produce a targeting vector. As noted by Good, this attempt *failed*. The second method is described in paragraphs [0123] to [0152], where Good describes use of PCR to amplify genomic DNA to produce a targeting vector. The vector that was constructed also *failed*, as described at paragraphs [0126] and [0152]. The third and final potential method for making a prion knockout cow described in Good is found in Example 2, which provides a *prophetic* procedure for constructing a targeting vector and producing a transgenic bovine. This third potential method was not carried out by Good and was not shown to result in the production of a prion knockout bovine. It is therefore indisputable that Good provides no experimental evidence, other than failures, with respect to attempts at producing a transgenic bovine lacking prion production.

1 As the Office was unable to locate this Declaration when preparing the present action, a courtesy copy is enclosed herewith. The Declaration was originally submitted on September 15, 2006.

Further, and consistent with the inadequacy of the Good disclosure, the application leading to the Good publication (09/816,546) and its child application (10/971,541) have consistently been rejected for lack of enablement by the U.S. Patent Office.[2] Despite a clear desire to overcome these rejections and a combined pendency of almost seven years, Good has never supplied to the Patent Office any experimental evidence that they can use their methods to produce even a transgenic, heterozygous cell line, much less a living bovine with reduced prion production. Furthermore, as has been noted in Declarations by both Dr. Kuroiwa and Dr. Robl, to the best of their knowledge, the current Applicants are the first and only group to produce a PrP knockout bovine.

Accordingly, there exists no evidence of record showing that the methods of Good would result in a PrP knockout bovine or cell, as instantly claimed. Nor is there any evidence that Good, using the methods provided in their specification, either at the time of filing or to date, can produce a transgenic bovine or bovine cell with reduced prion production.

Extrinsic Evidence

To overcome the § 102 rejection, Applicants have consistently argued that Good is not an enabling disclosure and therefore cannot anticipate the instant claims. To support this view, Applicants have submitted two previous expert declarations, which are reviewed below.

Declaration of Dr. Kuroiwa of September 2006

This Declaration, which was filed on September 15, 2006, focused on the inadequacies of the Good disclosure with respect to knockout vector construction. In this Declaration, Dr. Kuroiwa opined that Good provided insufficient information for producing a targeting vector without a substantial amount of experimentation involving a large degree of uncertainty. In particular, Dr. Kuroiwa opined that Good failed to provide

2 Child application 11/827,103 was filed on July 9, 2007 and is not currently publicly accessible.

any method that would result in isolation of the PrP genetic sequence necessary to construct a targeting vector with any certainty, especially given the failed attempts discussed above, and further failed to provide information on how to isolate the bovine PrP DNA in a form capable of being employed in a targeting vector. Based on this uncertainty, Dr. Kuroiwa also found Good lacking a sufficient disclosure on how to construct a targeting vector from the genomic sequence and on the form of the vector to be used in transfection. Thus, in Dr. Kuroiwa's view, Good failed to provide the skilled artisan with sufficient guidance for the production of a prion knockout bovine without undue experimentation.

Dr. Kuroiwa further explained the differences between Good and the present disclosure and detailed the teachings of Applicants' specification that would allow others to produce prion knockout cells and bovines. For example, the present specification describes the identification of a genomic PrP DNA; provides exemplary targeting vectors; methods for their construction; structural information on the vector; methods of animal cloning; and a diagnostic PCR to genotype the cells after drug selection. Furthermore, the present specification provides data showing that hemizygous and homozygous KO cells were actually produced using the described methods, and PrP knockout bovines have subsequently been produced using the methods described in the application. The detailed nature of the present specification highlights the inadequacies of the Good disclosure described by Dr. Kuroiwa.

Declaration of Dr. Robl of June 2007

As the Office maintained the rejection for anticipation over Good after submission of Dr. Kuroiwa's Declaration, Applicants submitted an additional Declaration from Dr. Robl providing yet further reasons why the methods of Good are inoperable. Dr. Robl's Declaration focused on the inadequacies of the Good disclosure with respect to the targeted colony selection and cloning steps. In this Declaration, Dr. Robl opined that Good taught a plating density (i.e., 500,000 candidate targeted cells per well) and an

isolation technique (i.e., cloning rings) that would be inoperable, because this density is too high to allow for efficient isolation of individual colonies. Colonies isolated using cloning rings, as taught in Good, would therefore be mixed, i.e., containing targeted and non-targeted cells. Dr. Robl further stated that while Southern blot genotyping on such a mixed colony would produce a positive signal, there is no guarantee that fetuses produced would be transgenic. Dr. Robl also found the proposed primary screening method (i.e., PCR followed by Southern blot) to be inoperable, as Good provides no teachings on how to obtain sufficient DNA from selected colonies to perform PCR followed by Southern blotting. In Dr. Robl's opinion, the targeted cells would senesce before a sufficient quantity of DNA was obtained to perform the proposed genotyping step. Finally, Dr. Robl found Good's guidance on the second targeting vector also to be insufficient. While Good proposed use of two targeting vectors, the reference failed to provide guidance on how to control for retargeting of the first allele by the second vector, a necessary step to produce a prion free bovine. As with Dr. Kuroiwa, Dr. Robl provided scientific reasoning to show that one skilled in the art could not make a PrP knockout cell or bovine using the methods of Good without undue experimentation.

Dr. Robl also provided an explanation of how the present specification allows one skilled in the art to actually produce living PrP knockout cells and bovines with particular reference to the inadequacies of Good. Dr. Robl noted that the plating density taught in Applicants' specification (7000 cells per well) allowed for isolation of individual colonies of targeted cells. Furthermore, the specification provided PCR-based screening methods for determining correctly targeted cells that require minimal population doublings. Finally, the present specification provides two targeting vectors and methods to determine whether the second vector correctly targeted the second allele.

Again, Dr. Robl's Declaration indicates that one skilled in the art could make a PrP knockout cell or bovine using the methods of the present specification, while highlighting the inadequacies of the methods described in Good.

Response to the Office's Arguments Regarding the Expert Declarations

In maintaining the present rejection, the Office alleges that there are three deficiencies in the expert declarations provided by Applicants: (1) that others have previously used cloning rings to isolate correctly targeted fibroblasts; (2) that production of a single correctly targeted cell is all that is needed to allow production of a living bovine; and (3) that selection of cells correctly targeted by a second vector could be accomplished using simultaneous selection against two antibiotics. Applicants again disagree and provide a further Declaration by Dr. Kuroiwa to address these specific contentions of the Office.

The first issue relates to the use of cloning rings to select targeted cells for cloned animal production. As discussed above, it is Dr. Robl's opinion that the selection method of Good would be inoperable because the plating density taught in Good (500,000 cells per well) and the use of cloning rings would result in mixed colonies of cells, and that, as a result, there would be no guarantee that fetuses produced using cells from this mixed colony would be transgenic. In response to this opinion, the Office has provided a reference by Bondioli as evidence of the successful use of cloning rings for colony selection. Applicants respectfully submit that this reference fails to support the Office's contention. Bondioli, in fact, actually supports Applicants' position, as the experiments described in the reference employed cloning rings and failed to isolated correctly targeted cells – exactly as earlier opined by Dr. Robl.

The Office is specifically directed to the presently submitted Kuroiwa Declaration. Dr. Kuroiwa states, "I have considered the statement by the Office, 'at the time of filing, transfected fetal fibroblasts had been isolated in the art by culturing transfected fibroblasts in the presence of selection medium followed by isolation with cloning rings ([Bondioli, et al. Mol. Reproduct. Develop., 2001, 60,] page 190. col. 1, parag. 2, lines 10-24),' and disagree with the Office's conclusions." As noted by Dr. Kuroiwa, "[t]he Bondioli reference *attempted* to introduce a puromycin resistance gene into fibroblasts from a transgenic boar (page 190, first column). The authors then used cells isolated

using cloning rings from puromycin-containing medium to produce cloned piglets (pages 190-191). PCR analysis of *the cloned piglets* indicated, however, that they *did not contain the puromycin resistance gene* (page 191, second column). Thus, *the authors of the reference failed to produce a correctly targeted pig using cells isolated by cloning rings*." (¶2, emphasis added) This, of course, is precisely the scientific problem raised by Dr. Robl – that use of cloning rings results in mixed colonies and, in the context of Good, does not allow for selection of targeted cells for animal production.

Dr. Kuroiwa further comments on this phenomenon, stating, "In general, it is known that selecting transgenic somatic cells by antibiotics does not always produce the expected transgenic cloned animals." This phenomenon is called the "bystander effect," where transgenic cells expressing an antibiotic-resistance gene provide protection to nearby non-transgenic cells either by secretion of the gene product into the medium or by direct cell-to-cell contact. As a result, Dr. Kuroiwa notes that "many transfected colonies are mixed and contain both transgenic and non-transgenic cells." (¶2). Thus, all evidence of record -- Dr. Robl's Declaration, the Bondioli reference supplied by the Office, and Dr. Kuroiwa's Declaration submitted herewith -- indicates that the plating method of Good would not be operable without undue experimentation.

The second issue raised by the Office relates to the number of targeted cells needed in practice to produce a cloned knockout animal. Dr. Kuroiwa has "considered the statement by the Office, 'all there needs to be [to produce a live cow] is one bovine fetal fibroblast containing a nonfunctional PrP gene, which can then be grown to produce a cell line.'" Dr. Kuroiwa also disagrees with this position, stating "As noted in my declaration filed on June 2007, ¶4, not all correctly targeted cell lines produce live offspring, even though they may produce pregnancies. In my experience, cloning efficiency for producing live animals is approximately 10%. Accordingly, one would typically require ten cell lines to produce a live animal. Thus, in my opinion, *neither I nor other scientists in this area would believe that a single, correctly targeted cell would be sufficient to produce live offspring*." (¶3, emphasis added).

Finally, with respect to the third issue of determining whether heterozygous cells have been correctly targeted to produce homozygous cells, Dr. Robl has previously opined that Good, while recognizing the problem, fails to address it sufficiently. In response, the Office has stated that "It is possible, that the second targeting vector will insert at the second prion locus. Thus, cells that are resistant to both antibiotics would have a reasonable expectation of having both loci targeted." To carry out the approach proposed by the Office, a double selection method would be necessary, in which double knockout cells are simultaneously selected for using media containing two different antibiotics. As noted by Dr. Kuroiwa, in practice, this technique would not work in a reproducible fashion because of the nature of primary somatic cells. Dr. Kuroiwa states, "In general, somatic cells, like transgenic fibroblasts, are fragile. I am not aware of anyone using simultaneous selection against two antibiotics for selection of somatic cells in animal cloning. In my opinion, such a selection process would be unsuccessful." (¶4). Applicants also note that the Office has not provided any evidence to support its assertion that a double screening procedure could be employed during cloning with somatic cells.

Applicants again assert that for a number of reasons the Good disclosure is not enabling as evidenced by the Robl and Kuroiwa expert declarations. These declarations continue to go unchallenged by contrary scientific evidence.

The Legal Standards for an Enabling Anticipatory Disclosure

Applicants further disagree with the Office on the standards for enablement of an allegedly anticipatory reference. As the Federal Circuit has recently stated: "invalidity based on anticipation requires that the assertedly anticipating disclosure [enable] the subject matter of the reference and thus of the patented invention without *undue experimentation*." *Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research*, 346 F.3d 1051, 1052 (Fed. Cir. 2003; emphasis added). Thus, the question is not whether, if one attempted the methodology of Good indefinitely, one could eventually produce a PrP knockout bovine. Nor is the standard for non-enablement that the methods of Good must

never result in a transgenic bovine. Rather, the question is whether Good provides to the public a method for producing a PrP knockout bovine without undue experimentation. As discussed above, Applicants have provided ample evidence that Good does not meet this standard.

Moreover, the Office has supported its anticipation rejection by supplying technical information and approaches not found in Good. On this point, the Federal Circuit has held that a reference is enabled only if "one of ordinary skill in the art could have combined the publication's description of the invention with his own knowledge to make the claimed invention." *Id.* at 1055. While other references may be consulted, reliance on such references is necessarily limited for an anticipation rejection. Anticipation requires that all of the limitations of a claim be found in a *single* prior art reference. *Scripps Clinic and Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 1576 (Fed. Cir. 1991). Even so, the courts have acknowledged: "It is sometimes appropriate to consider extrinsic evidence to explain the disclosure of a reference." *Id.* Importantly, "[t]he role of extrinsic evidence is to educate the decision-maker to what the reference meant to persons of ordinary skill in the field of the invention, not to fill gaps in the reference." *Id.* Stated another way, "extrinsic evidence may be considered when it is used to explain, but not expand, the meaning of a reference." *In re Baxter Travenol Labs.*, 952 F.2d 388, 390 (Fed. Cir. 1991) (citing *Scripps*, 927 F.2d at 1576-1577). Accordingly, the Office may only rely on references extrinsic to Good when they explain the meaning of Good or the knowledge of one skilled in the art, but not to replace the teachings of Good with alternate methodologies. That is, if the skilled artisan is required to ignore explicit teachings of Good and to replace them with other methods, Good is not anticipatory.

Based on this standard, the Office's reliance on Dai and Zheng is misplaced for an anticipation rejection. Good states that the cells in its methods would be near senescence after clonal expansion (¶ 171). Based on this statement, Dr. Robl found the teachings of Good lacking for production of sufficient DNA for Southern blotting. While Applicants

acknowledge that Dai employed Southern blotting in cloning, the Office has not shown that Good was employing the methods of Dai to produce targeted cells. Thus, the ability of Dai to produce enough cells for Southern blotting, which Applicants note was only successful for ~ 50 % of colonies (page 252, second column), does not *explain* the disclosure of Good. Employing the methods of Dai or modifying the methods of Good to produce transfected cells is instead an expansion of the disclosure of Good, which is not proper for an anticipation rejection. The Office further argues that at the time of filing other methods for identifying properly targeted fibroblasts were known, citing Zheng. Good, however, expressly provides a screening method for its targeted fibroblasts, and this method requires both PCR and Southern blotting. Thus, any reliance by the Office on techniques other than those taught, and presumably required, by Good also goes beyond merely explaining Good and instead results in an improper expansion of the disclosure. Dai and Zheng may not be used to expand the disclosure of Good, if Good is to be used as an anticipatory reference. Further, Applicants submit that citation of these references by the Office highlights the non-enabling nature of the Good disclosure.

On the sufficiency of Applicants' evidence of non-enablement, the Office asserts: "What applicant needs to provide is evidence, arguments or reasoning that the method of Good would never provide a homozygous prion knockout fibroblast." This position of the Office is inconsistent with case law and M.P.E.P. § 2121.02. In *In re Hoeksema*, the court considered whether an Applicant had provided sufficient evidence regarding the operability of a prior art reference. *In re Hoeksema*, 399 F.2d 269 (C.C.P.A. 1968). In that case, the court held that an expert affidavit was sufficient evidence of inoperability, and that proof that a disclosure could *never* enable an invention was not the standard. *Id.* at 275. Rather, the court stated that "It would be practically impossible for an applicant to show that all known processes are incapable of producing the claimed compound." *Id.* (footnote 9). As in *Hoeksema*, here, Applicants have provided numerous declarations to the effect that Good is not enabling, and, as is detailed above, the Office has not provided evidence that disputes Applicants' assertions. Applicants have met their evidentiary

burden to show that Good is not enabled; proof that Good could never work given infinite time and resources is not required.

Finally, the Office also objects to the present claims as "not enabled as they encompass those of Good." Applicants again disagree with this legal standard. M.P.E.P. § 2164.08(b) makes it clear that: "The presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art...." Thus, the fact that a claim covers an inoperative embodiment is not dispositive on the issue of enablement.

Moreover, M.P.E.P. § 2164.08(b) states: "typically, inoperative embodiments are excluded by language in a claim (e.g., preamble)..." This is also true in the present case. Applicants' position argued in both previous replies is that the present claims do not encompass the methods of Good, because non-working embodiments are excluded by the claim language. Claim 1 requires a bovine, and claim 25 requires an isolated cell, each having reduced prion production. These claims therefore require an animal or a cell that has been successfully produced, and not a failed attempt. Good's inoperative embodiments are excluded. In addition, claims 32 and 37 cover methods for producing an isolated transgenic bovine or bovine cell having reduced expression of functional prion protein. Again, Good's methods would not successfully produce such bovines or bovine cells. Accordingly, the preambles of claims 32 and 37 as well exclude the inoperable embodiments (i.e., the methods) of Good, consistent with U.S. patent practice as outlined in the M.P.E.P.

Potential for Interference

Finally, Applicants acknowledge the potential for an interference between the present application and a child application of Good. Should a family member of Good ever be in condition for allowance and include claims interfering with the present claims,

Applicants note that the interference proceedings would allow both sides to provide evidence as to which party first conceived of the invention and whether the applications as filed properly support such claims under 35 U.S.C. § 112. Applicants, however, disagree with the Office's proposed procedure of suspending prosecution of the present case until such time as a decision is reach on enablement of the Good specification. Applicants note that, to date, rather than presenting evidence of enablement, Good has now twice abandoned an application in favor of a continuation application. In contrast, Applicants have shown the production of living PrP knockout bovines. Based on M.P.E.P. § 2303.01, Applicants submit that the proper procedure for these two applications is to issue the present application and await the completion of examination in the Good family before considering the propriety of an interference (see Example 2 of M.P.E.P. § 2303.01). Applicants request reconsideration on this issue.

Rejections under 35 U.S.C. § 103

Claims 1 and 7 stand further rejected for obviousness over Good in view of Muramoto, and claims 25, 31, 37, and 38 stand rejected for obviousness over Good in view of Collas. Both of these rejections rely of the Office's position that Good anticipates the teachings of claim 1. As discussed above, Good fails to anticipate claim 1 because it is inoperable. Furthermore, the teachings of Muramoto on amber mutations in prion genes and the teachings of Collas on permeabilized cells do not remedy the deficiencies of Good with respect to enablement of the fundamental process of producing PrP knockout cells or bovines.

In addition, with respect to Muramoto, Applicants note above that the reference teaches that the introduction of the same mutation, i.e., a STOP codon, into two different mammals produced two different results. Accordingly, if anything, the Muramoto reference would point scientists to attempt a prion knockout in bovines another way.

The § 103 rejections may also be withdrawn.

Cited References

The Office relied on the Zheng reference in the previous action, but this reference was not listed on the accompanying Form 892. Applicants request that the Zheng reference be listed on a Form 892 and accompany the next action.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. Enclosed is a petition to extend the period for reply for three months, to and including February 11, 2008, February 9th being a Saturday. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: February 11, 2008

Karen L. Elbing, Ph.D.
Reg. No. 35,238

J. Cooper McDonald, Ph.D.
Reg. No. 52,011

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045